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Relaxant Effects of *Curcuma aeruginosa* Rhizome Extracts on Isolated Rat Gastric Fundus and Ileum Contraction

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Abstract

Curcuma aeruginosa (CA) rhizomes are used in traditional medicine as gastrointestinal remedies, postpartum care and uterine issues. However, its effects on gastrointestinal motility have not yet been explored. Extracts of CA rhizomes prepared using chloroform, methanol and water were evaluated for their effects on gastrointestinal contractions using isolated rat gastric fundus and ileum. Varied solvents aim for extraction of different compounds of CA rhizome. In the ileum, the 3 extracts did not significantly affect spontaneous contractions, but significantly reduced contractions induced by acetylcholine (ACh, 0.3 μM) and KCl (40 mM) in a concentration-dependent manner. The order of potency, based on ACh-induced contraction, was chloroform > methanol > water extracts, with respective IC_{50} values of 0.025 ± 0.001, 0.46 ± 0.02 and 2.57 ± 0.47 mg/mL. Similar potency order against KCl-induced contraction was observed, with IC_{50} values of 0.011 ± 0.002, 0.47 ± 0.06 and 1.18 ± 0.18 mg/mL, respectively. In the isolated gastric fundus model, both methanol and water extracts resulted in an increase in the force of contraction in the absence of stimulation. However, when ACh (0.3 μM) was introduced, the methanol and water extracts exhibited a concentration-dependent reduction in contractions, with IC_{50} values of 0.62 ± 0.06 and 2.30 ± 0.32 mg/mL, respectively. As for KCl (40 mM)-induced contraction, only the highest concentration of the water extract (3.75 mg/mL) significantly decreased the contraction by 41.29 ± 2.94 %. CA extract demonstrated relaxant effects on both isolated rat gastric fundus and ileal contractions induced by ACh and KCl. These findings support the use of CA in traditional medicine as a gastrointestinal remedy. These extracts have the potential to be used as antispasmodics and to treat infectious and non-infectious diarrhea, the latter of which occurs in functional gastrointestinal diseases. It might also be useful as a gastroprokinetic.

Keywords: *Curcuma aeruginosa*, Gastric fundus, Gastrointestinal remedies, Ileum contractions, Rhizome extract

Introduction

Curcuma aeruginosa Roxb. (CA) rhizome holds a worthy place in traditional medicine given its application in gastrointestinal remedies, postpartum care and uterine issues. However, the specific effects of CA on gastric and intestinal functions remain largely unexplored. Therefore, there is a crucial need for comprehensive evidence to support the safe and efficacious use of CA in the management of gastrointestinal conditions.

Curcuma aeruginosa Roxb. (CA, family *Zingiberaceae*) is a tropical plant native to Southeast Asia. In Thai, it is commonly referred to as "Waan-Maa-Haa-Mek" or "Kajeawdang." This perennial plant has green shoots, oblong roots and a height of 45 - 60 cm. It is characterized by greenish-blue or bluish-black rhizomes, a purple calyx, a dark purple leaf sheath and red corolla lobes [1,2]. The CA rhizome has been long used in traditional medicine for gastrointestinal remedies, for diarrhea and colic and postpartum care for women, including uterine involution, pain relief and inflammation management [2,3]. Moreover, extensive research has highlighted the various pharmacological properties of CA, such as its potential as an anticancer, antioxidant, antimicrobial, anti-dengue, immunostimulant, anthelmintic, anti-inflammatory, antiandrogenic, antinociceptive and antipyretic agent [4]. The uterine relaxant action of CA extracts may be attributed to the presence of β-pinene and certain sesquiterpene lactones [3]. Notably, the chloroform

extract exhibits greater potency than that of the methanol extract [3]. A total of 34 phytochemicals were identified in the rhizome of CA, including 3 diarylheptanoids, 26 terpenoids and 5 flavonoids. The key bioactive substances included cycloisolongifolene, curzerenone, 1,8-cineole, 8,9-camphor, dehydro-9-formyl, curcumenol and germacron. The essential oil derived from both the rhizomes and leaves of this plant constitutes approximately 0.32 % and primarily consists of steroids, esters, monoterpenes (e.g., camphor, α-pinene, β-pinene and 1,8-cineole) and sesquiterpenes (e.g., germacrone and curzerene). Furthermore, CA essential oils demonstrated efficacy against *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans* [5]. Additionally, they have exhibited antimicrobial activity against other bacteria*, such as Escherichia* coli and *Vibrio cholera*, causing diarrhea [4]. It may also be useful as a medicinal herb to treat functional gastrointestinal disorders (FGIDs).

Chronic disorders known as FGIDs are characterized by recurrent and persistent gastrointestinal symptoms. The term "brain bowel interaction disorders" is increasingly being used to describe these diseases. Functional diarrhea (FDr) and irritable bowel syndrome with diarrhea (IBS-D) are the 2 most prevalent functional bowel illnesses. According to the Rome IV criteria, the fundamental difference between these 2 conditions is the presence of abdominal pain. Abdominal pain must have been present for an average of 1 week in the previous 3 months for the diagnosis of irritable bowel syndrome (IBS). Abdominal pain should not be the primary complaint in patients with FDr, despite the possibility of its occurrence. Loperamide is the first-line treatment [6].

FGIDs are widely distributed worldwide, affecting more than 40 % of people globally [7]. People from different nations use herbal medicines as prescription or at-home remedies. People with FGIDs can use them as complementary and alternative medicine when conventional treatments are unsuccessful, and treatment guidelines for FGIDs recommend such therapies in some countries [8].

Peppermint oil (PMO) is one example of a natural remedy for FGIDs. Multiple lines of evidence suggest that PMO functions as a smooth muscle relaxant in the GI tract. PMO and its component menthol exhibit calcium channel blocking actions in guinea pig ileal smooth muscle *in vitro*, which aids in the relaxation of the intestinal smooth muscle. Additionally, PMO significantly reduced contractions in the taeniae coli of guinea pig induced by acetylcholine, histamine, 5-hydroxytryptamine and substance P [8].

CA has long been used as a food or traditional medicine for the treatment of many illnesses. Previous studies have indicated that chloroform and methanol extracts of CA possess uterine relaxant effects in isolated rat uteri, consistent with their traditional medicinal uses [3]. There is the potential to be an effective gastrointestinal remedy. However, the pharmacological effects of CA on the gastrointestinal system remain unexplored and require further investigation. The present study aimed to investigate the effect and plausible mechanism of action of plant extracts on gastrointestinal motility using isolated rat gastric fundus and ileum. Experiments were performed on spontaneous, acetylcholine- and potassium chloride-induced contractions.

Materials and methods

Plant material and extraction procedure

Fresh CA rhizomes were carefully collected from Songkhla Province, Thailand. The botanical identification of the specimens (voucher number: SN 4601010) was performed by Assoc. Prof. Dr. Sanan Subhadhirasakul (Prince of Songkla University), an expert in the field. To preserve authenticity, the verified specimens were safely housed at the esteemed herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

Chopped and dried CA rhizomes were sequentially extracted using chloroform and methanol. For chloroform extraction, the plant material was soaked in chloroform for 7 days at room temperature, followed by filtration. The maceration process was repeated 4 times and the resulting extracts were combined. The chloroform was then evaporated under reduced pressure using a vacuum rotary evaporator to obtain a chloroform extract (81.5 g; yield 4.28 %). To prepare the methanol extract, the plant residue remaining after the chloroform-based extraction was dried at room temperature overnight. The dried residue was extracted with methanol using a previously described method. Methanol was then removed from the extract using the same equipment (122.1 g; yield 6.43 %).

Additionally, separate extractions using boiling distilled water were performed on another batch of chopped and dried CA rhizomes. The plant material was boiled in distilled water for an hour, and a water extract was obtained by filtration. The extraction process was repeated twice, and the combined water extract was evaporated under reduced pressure to yield the water extract (274.4 g; yield, 7.97 %). The extracts were stored at 4 °C until further use.

Drugs and chemicals

The pharmacological agents employed in this study were acetylcholine perchlorate (ACh), atropine sulfate, loperamide hydrochloride and verapamil hydrochloride (Sigma, St. Louis, USA). They were dissolved in distilled water. The water extract of CA rhizome was also dissolved in distilled water, whereas the methanol and chloroform extracts of CA rhizome were dissolved in dimethyl sulfoxide (DMSO) to achieve a final concentration of 200 mg/mL and stored as stock solutions at 4 °C until further use. The final DMSO concentration in the organ bath was maintained at 0.5 % (v/v). The Krebs-Henseleit (Krebs) solution used in the experiments consisted of the following concentrations (in mM): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; KH_2PO_4, 1.03; $MgSO_4$, 0.45; $CaCl_2$, 2.5; D-(+)glucose, 11.1; disodium edetate, 0.067; and ascorbic acid, 0.14.

Animals and ethical approval

Male and female Wistar rats weighing 200 - 250 g were supplied by the Southern Laboratory Animal Facility, Prince of Songkla University, Hat Yai Campus, Songkhla, Thailand. The animals were housed in a controlled environment in an air-conditioned room maintained at a temperature range of 24 - 26 °C and a humidity level of 50 %. A standard 12-h light/dark cycle was used to regulate the daily rhythm.

Throughout the experimental period, the rats had ad libitum access to rodent laboratory chow and water. All animal handling and care procedures strictly adhered to the ethical guidelines. The study protocol was thoroughly reviewed and approved by the Ethics Committee on Animal Experiments, Prince of Songkla University, Thailand (approval no. 24/2553).

Preparation of isolated rat gastric fundus and ileum

The experimental procedures for tissue preparation were adapted from the established methods employed by the Staff of the Department of Pharmacology at the University of Edinburgh in 1970. Rats were euthanized by cervical dislocation, followed by exsanguination. Subsequently, the abdomen was meticulously opened, and the entire stomach and intestines were carefully excised.

The fundus section of the stomach, characterized by its distinctive gray coloration, was meticulously separated from the pyloric part, which was distinguished by a pink color. To create a fundus strip, the fundus was longitudinally opened, placed in a dish containing Krebs solution, and made into a strip approximately 4 - 5 cm in length and 0.3 cm in width through a transverse cut.

To prepare the ileal segment, a section above the ileocecal junction was dissected and subsequently divided into segments measuring 2 cm long. In case of the presence of residual food material in the ileum, it was gently expelled by the delicate introduction of Krebs solution (37 °C) into the lumen using a Pasteur pipette.

Following meticulous tissue preparation, the fundus or ileum segments were carefully positioned in an organ bath containing 20 mL of Krebs solution, which was continuously aerated with 95 % O_2 and 5 % CO_2. Tissue preparations were loaded with 2 g of tension, and a crucial equilibration period of 30 min was allowed prior to commencing the experiments. Throughout the equilibration phase, the tissues were washed with fresh Krebs solution every 10 min to ensure a stable and conducive physiological environment.

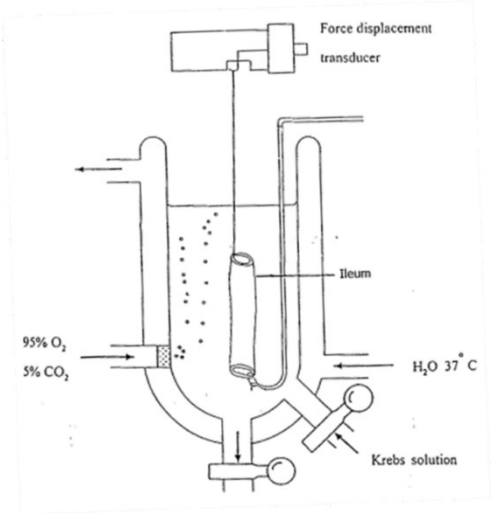

Figure 1 Set up of isolated ileum preparation in a double-jacketed organ bath for recording of its contraction (Reprinted from Poonpanang [9]).

The contractile responses of the fundus and ileum segments were meticulously recorded isometrically using a force FT03 displacement transducer connected to a reputable Grass Model 7H polygraph (Grass International Co., Quincy, Mass, USA) (**Figure 1**). This precise and reliable recording method allowed for the accurate measurement of tissue contraction during the experimental procedures. The contraction force was determined by measuring the height of maximum contraction in response to either ACh or KCl.

Effect of extracts on ileum contraction

This study investigated the effects of CA rhizome extract on spontaneous ileal contractions. After a 30-minute equilibration period, the CA extract was introduced into the bathing solution at a single concentration. Ileum contractions were recorded to assess the effects of the CA extract. Additionally, a concurrent control experiment was conducted to investigate the effects of dimethyl sulfoxide (DMSO) on ileal contraction.

The effect of CA rhizome extracts on ACh-induced ileum contractions was investigated as follows. Initially, the rat ileum was allowed to equilibrate for a 30-minute period. Subsequently, contraction was induced using a submaximal concentration of the spasmogen ACh (0.3 μM). The ileum was subsequently washed with fresh Krebs-Henseleit solution every 10 min until it returned to its resting level. This process was repeated 2 or 3 times until a stable response to ACh was obtained. Next, the effects of the plant extract and loperamide were determined in a single-concentration manner. This was achieved by pre-incubating the ileum strip with a single concentration of the extract or drug for 15 min before the addition of ACh (0.3 μM). These steps were repeated with a higher concentration of the plant extracts. Additionally, the effects on ACh-induced ileum contraction were studied using the standard antidiarrheal drug loperamide, as well as a muscarinic receptor antagonist, atropine (1 μM). In a parallel control experiment, the effects of equal amounts of solvent vehicle on ACh-induced contraction**s** were assessed.

In addition, the effect of the CA rhizome extract on KCl-induced ileal contractions was investigated. The effects of the plant extracts were evaluated using a single concentration pattern on a KCl (40 mM)-precontracted ileum strip in the same manner as described for the study of ACh-induced contraction. The effects of the same quantities of solvent vehicle on KCl-induced contractions were assessed in a comparable control experiment. Furthermore, the antagonizing effect of verapamil **(**0.01 mM**)**, an L-type calcium channel blocker, was observed in KCl-induced contractions.

Effect of extracts on fundus contraction

The effects of CA rhizome extract on spontaneous gastric fundus contractions and ACh- and KCl-induced gastric fundus contractions were determined. After a 30-minute equilibration period, the effects of the extracts on the spontaneous contraction of the isolated rat fundus were assessed using the same method described above for the ileum. Additionally, following another 30-minute equilibration period, the effects of the extracts on the isolated gastric fundus, pre-contracted with either ACh (0.3 μM) or KCl (40 mM), were studied in the same manner as described for the isolated ileum.

Statistical analysis

Data are expressed as mean ± standard error of the mean**.** For each group, log concentration-response curves were plotted and regression lines were fitted to the linear portions of the curves to determine the IC_{50} values. Differences between means were analyzed using either an unpaired t-test or analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) test to determine individual differences. In some cases, analyses were performed on individual IC_{50} values obtained from each concentration-response curve for the test agent. A probability value **(***p***)** of less than 0.05 was considered indicative of statistical significance.

Results and discussion

Effects of the extracts on spontaneous, ACh- and KCl-induced contractions of rat isolated ileum

None of the extracts of CA rhizome, including the chloroform extract (0.005 - 0.1 mg/mL), methanol extract (0.1 - 1 mg/mL) and water extract (0.31 - 3.75 mg/mL), had a significant effect on the spontaneous contraction of rat isolated ileum.

The effects of CA rhizome extracts on the ACh-induced contraction of isolated rat ileum were compared to those of loperamide. The 3 plant extracts - chloroform extract (0.005 - 0.1 mg/mL), methanol extract (0.1 - 1 mg/mL) and water extract (0.31 - 3.75 mg/mL) - along with loperamide (0.3 - 10 μg/mL), produced concentration-dependent inhibition of ACh (0.3 μM)-induced contractions. A typical trace of this inhibition is shown in **Figure 2**. The IC_{50} values for the chloroform, methanol and water extracts were 0.025

± 0.001, 0.46 ± 0.02 and 2.57 ± 0.47 mg/mL, respectively, in the ACh (0.3 µM)-induced contraction model (**Figure 3(A)**).

Among the extracts, the chloroform extract was the most potent, outperforming the methanol and water extracts by approximately 18 and 103 times, respectively. The 3 plant extracts were much less potent than loperamide, with the IC_{50} of loperamide on ACh-induced contraction being 2.34 ± 0.14 µg/mL. At the highest concentrations used in this study, the inhibitions produced by the chloroform extract (0.1 mg/mL), methanol extract (1 mg/mL), and water extracts (3.75 mg/mL) were 91.75 ± 0.47, 75.83 ± 8.48 and 52.41 ± 14.50 %, respectively. Atropine (1 µM) completely abolished the ACh-induced contraction.

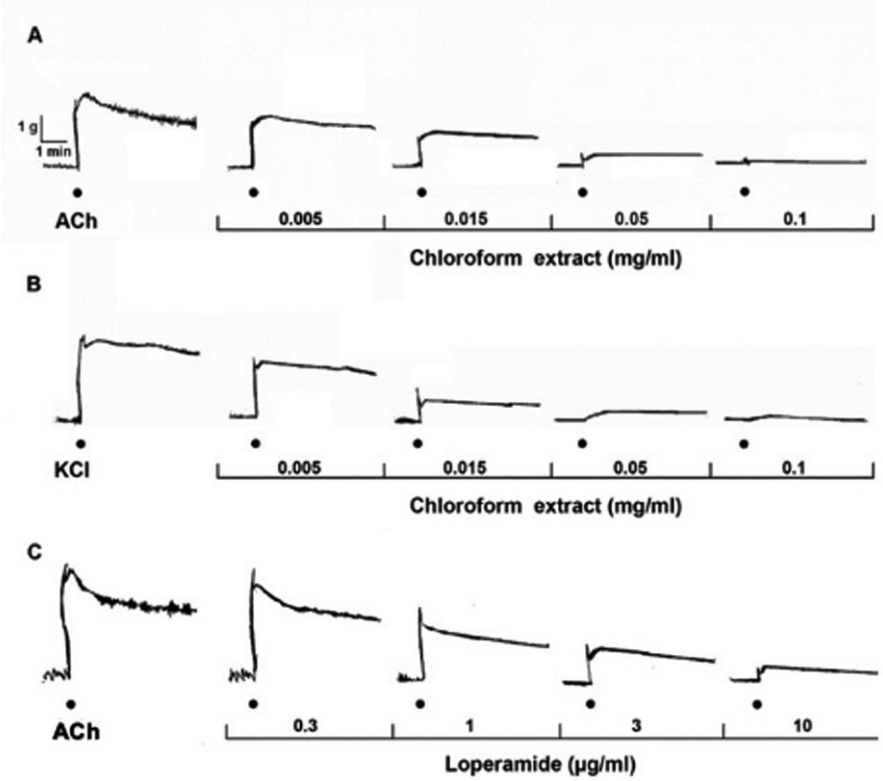

Figure 2 Typical recordings illustrate the effects of the chloroform extract of CA on the contractions of rat isolated ileum, induced by (A) ACh (0.3 µM) and (B) KCl (40 mM), along with the effect of loperamide on the contraction induced by (C) ACh (0.3 µM).

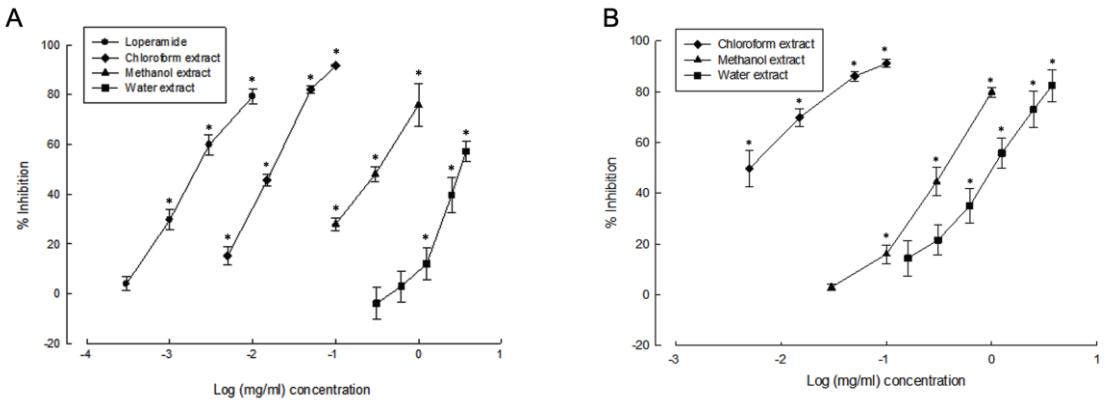

Figure 3 (A) Illustrates the inhibition of ACh-induced contraction of rat isolated ileum by loperamide (0.3 - 10 µg/mL), chloroform extract (0.005 - 0.1 mg/mL), methanol extract (0.1 - 1 mg/mL) and water extract (0.31 - 3.75 mg/mL) of CA rhizome. (B) Depicts the inhibition of KCl-induced contraction of rat isolated ileum by chloroform extract (0.005 - 0.1 mg/mL), methanol extract (0.03 - 1 mg/mL) and water extract (0.16 - 3.75 mg/mL) of CA rhizome. Symbols represent means, and vertical lines indicate the standard errors of the means (n = 5 - 10). Asterisks denote a significant difference from the control, with $p < 0.05$.

The effects of CA extract on KCl-induced contraction of isolated rat ileum were similar to those on ACh-induced contraction of the isolated ileum. The 3 plant extracts also elicited concentration-dependent inhibition of contraction induced by KCl (40 mM), as shown in **Figure 3(B)**. A typical example of this effect is shown in **Figure 2**. The IC_{50} values for the chloroform extract, methanol extract and water extract were 0.011 ± 0.002, 0.47 ± 0.06 and 1.18 ± 0.18 mg/mL, respectively, in the KCl (40 mM)-induced contraction model. The chloroform extract was approximately 43 and 107 times more potent than the methanol and water extracts, respectively. The inhibitions produced by the highest concentrations of the chloroform extract (0.1 mg/mL), methanol extract (1 mg/mL) and water extract (3.75 mg/mL) were 91.16 ± 1.62, 79.87 ± 1.93 and 57.23 ± 3.87 %, respectively. However, the KCl-induced contractions were completely abolished by verapamil (0.1 mM).

Effects of the extracts on spontaneous, ACh- and KCl-induced contractions of rat isolated gastric fundus

After a 30-minute equilibration period, spontaneous contraction of the gastric fundus strip disappeared. At the highest concentration of the methanolic extract (1 mg/mL) and at the 3 highest concentrations of the water extract (1.28 - 3.75 mg/mL), there was a concentration-dependent increase in the force of gastric fundus contraction. The results are shown in **Figure 4**.

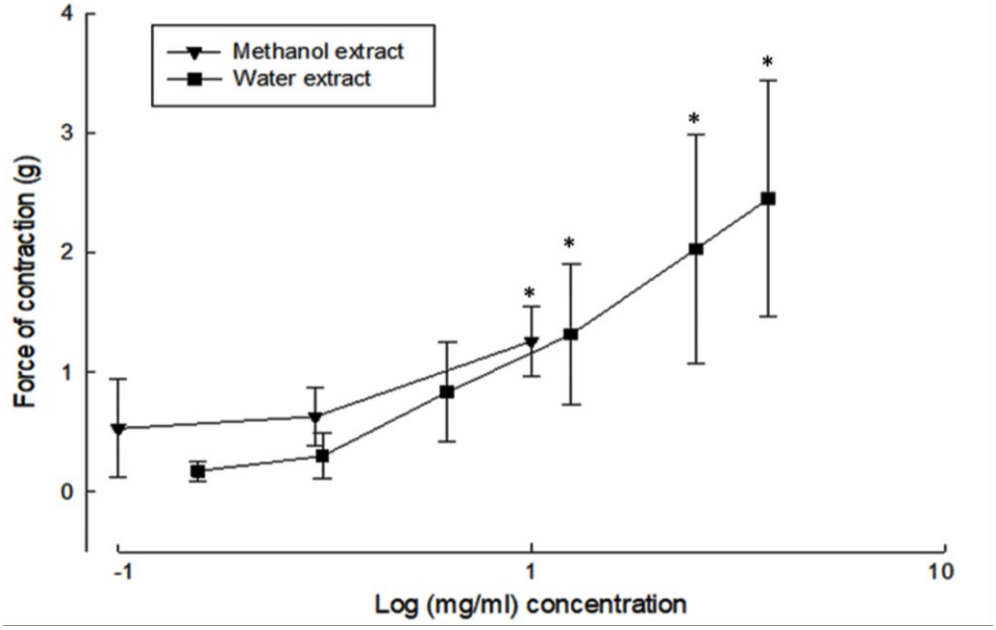

Figure 4 Force of contractions produced by CA methanol and water extracts on spontaneous contraction of rat fundus strips. Symbols represent means, and vertical lines indicate the standard errors of the means ($n = 6$). Asterisks denote a significant difference from the control, with $p < 0.05$.

The effects of the CA extract on ACh-induced contraction of isolated rat gastric fundus were investigated within specific concentration ranges. A typical trace of this inhibition is shown in **Figure 5**. The CA methanol extract (0.1 - 1 mg/mL) and the water extract (0.16 - 3.75 mg/mL) produced concentration-dependent inhibition of ACh (0.3 µM)-induced contraction, as shown in **Figure 6(A)**. The methanol extract was approximately 3.7 times more potent than the water extract. The IC_{50} values for the methanol and water extracts of CA rhizome on the inhibition of ACh-induced contractions of rat gastric fundus were 0.62 ± 0.06 and 2.30 ± 0.32 mg/mL, respectively. At the highest concentrations used in this study, 1 mg/mL for methanol and 3.75 mg/mL for water extracts, the inhibitions were 67.28 ± 6.69 and 73.35 ± 8.40 %, respectively. Nevertheless, the ACh-induced contraction was completely abolished by atropine (1 µM).

The effects of CA extract on KCl-induced contraction of rat isolated gastric fundus demonstrated that the water extract (0.16 - 2.5 mg/mL) did not significantly affect the KCl (40 mM)-induced contraction of the rat isolated gastric fundus. However, the highest concentration of the extract (3.75 mg/mL) significantly decreased the contraction by 41.29 ± 2.94 % (as shown in **Figure 6(B)**). Nevertheless, the KCl-induced contraction was completely abolished by verapamil (10 µM).

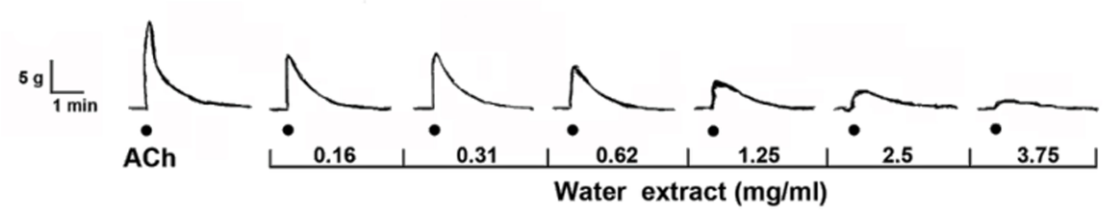

Figure 5 Typical recordings of the effects of the water extract of CA on the contractions of rat isolated gastric fundus, induced by ACh (0.3 μM).

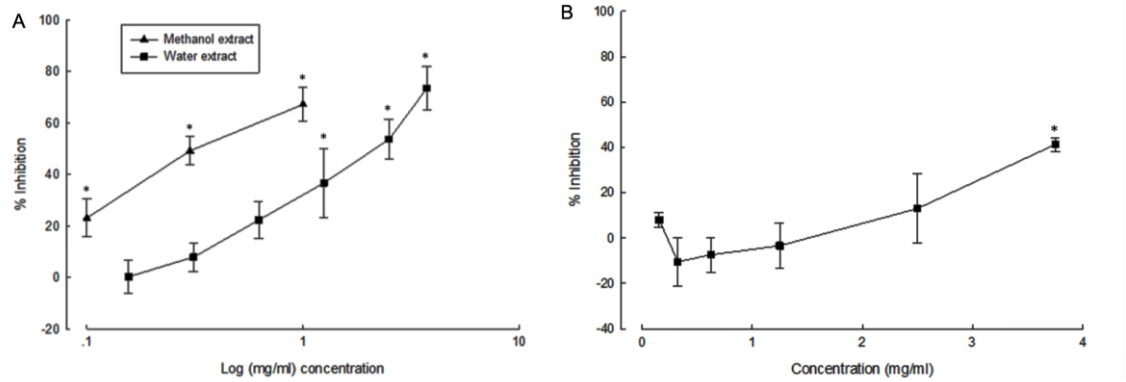

Figure 6 (A) Inhibition of acetylcholine-induced contraction of rat isolated gastric fundus by methanol extract (0.1 - 1 mg/mL) and water extract (0.16 - 3.75 mg/mL) of CA rhizome. (B) Effects of CA rhizome water extract on KCl-induced contraction of isolated rat gastric fundus. Symbols represent mean values, and vertical lines depict the standard errors of the means (n = 5). Asterisks (*) indicate values significantly different from the control at $p < 0.05$.

Acetylcholine may induce intestinal contraction by activating muscarinic receptors (M_3, M_2 and M_2/M_3 receptors), which leads to an increase in intracellular calcium concentration ($[Ca^{2+}]_i$). This increase may be due to the release of calcium from the intracellular stores. The primary cause of the increase in $[Ca^{2+}]_i$ is the influx of extracellular Ca^{2+}. This influx occurs due to the opening of nonselective cation channels (such as TRPC4 and/or TRPC6), which cause membrane depolarization and activate L-type Ca^{2+} channels [10-13].

Exposure of the intestinal smooth muscle to high K^+ solutions results in membrane depolarization, leading to the opening of voltage-dependent L-type calcium channels (VDCCs). This causes an increase in Ca^{2+} influx, and consequently, muscle contraction [14,15]. Later evidence showed that verapamil, a calcium channel blocker, completely abolished high K^+-induced intestinal contractions. An increase in intracellular Ca^{2+} due to membrane depolarization leads to calcium-induced calcium release (CICR) from ryanodine receptors (RYRs). Additionally, voltage-dependent conformational changes in VDCCs can directly open RyRs and IP3 receptors without any Ca^{2+} influx. The latter occurs via phospholipase C (PLC) activation [16].

In this study, the CA rhizome extracts inhibited both ACh (pharmacochemical coupling) and KCl (electrochemical coupling)-induced ileal contractions (**Figure 2**). These contractions were fully suppressed by a muscarinic receptor antagonist, atropine (1 μM) and a voltage-gated L-type calcium channel blocker, verapamil (0.1 mM), respectively. Our findings align with those from previous studies, in which ACh-induced ileal contractions were almost entirely blocked by verapamil [17-19]. Therefore, it can be conjectured that CA extract might inhibit ileal contractions by interrupting the influx of Ca^{2+}, likely through voltage-gated L-type calcium channels. Several evidence have demonstrated using both *in vitro* and *in vivo* models that the phytochemical compounds in plants, e.g., flavonoids and volatile oils, have been shown to have spasmolytic effects by blocking calcium influx through L-type calcium channels [20].

It has been reported that the rhizome of CA grown in Thailand contains essential oils such as β-pinene (7.71 %), 1,8-cineol (9.4 %) and cuzerenone (41.63 %) [21]. The chloroform extraction of CA logically yields these essential oils because of their similar polarities. α-Pinene and β-pinene, or the *Ferula gummosa* extract containing these compounds, displayed spasmolytic activity on ACh- or KCl-induced contractions of isolated rat ileums [22]. Moreover, evidence has shown that rat gastric strips contract *in vitro* to both α- and β-pinene, but relax the duodenum [23]. Recently, 1,8-cineol was found to significantly inhibit the

spontaneous contractions as well as the contractions induced by spasmogenic substances ($BaCl_2$, KCl and carbachol) of the bovine ileums [24]. As these effects are antagonized by verapamil, it suggests that 1,8-cineol blocks voltage-dependent calcium channels [24]. However, no evidence has been found regarding the effects of cuzerenone on smooth muscle contraction. Thus, it is conceivable that α-pinene, β-pinene and/or 1,8-cineol may play a significant role in the spasmolytic activity of the CA extracts on isolated rat ileum. Our findings support the traditional use of this plant for its antidiarrheal and antispasmodic properties.

Other components have also been reported in the rhizome of CA, including 3 guaiane sesquiterpene lactones, zedoalactone A, zedoalactone B and zedoarondiolextracted from the *n*-butanol fraction [25]. Additionally, 6 sesquiterpenes, including isofuradiene, furanodienone, dehydrocurdione, curcumenone, 13-hydroxygermacrone and zedoarol, have been isolated from the chloroform extract of CA [26]. The sesquiterpene lactones found in CA rhizomes may have effects similar to those of a sarcoplasmic reticulum Ca^{2+} ATPase inhibitor. For example, thapsigargin, a sesquiterpene lactone extracted from *Thapsia garganica*, inhibits the oxytocin-induced contraction of myometrium [27]. Dehydrocurdione, a sesquiterpene isolated from *Curcuma zedoaria*, inhibits the contractile responses of guinea-pig ileum to ACh (0.01 - 10 μM) or histamine (0.03 - 1 μM) [28]. It significantly reduced the high-K^+-stimulated increase in cytosolic Ca^{2+} levels in Fura-2-loaded rat mesenteric arteries. Thus, the inhibitory effects of dehydrocurdione on intestinal and vascular smooth muscles may be mediated by blocking Ca^{2+} entry from the extracellular space. As previously mentioned, dehydrocurdione is also present in CA rhizome [26]. Mono- and sesquiterpenes, such as essential oils extracted from *Zingiber roseun* (e.g., β-pinene and caryophyllene), have been shown to relax both carbachol- and KCl-induced contractions of isolated rat duodenum [29]. Therefore, α-pinene, β-pinene and sesquiterpenes might contribute to the rat intestinal relaxation effects observed for CA in the present study.

Our results demonstrated that the chloroform and methanol extracts exhibited higher potency than the water extract, likely because they contained a more substantial amount of β-pinene, 1,8-cineol and sesquiterpenes. These terpene compounds possess very low solubility in water, but are soluble in alcohol, ether, benzene and chloroform. In the extraction sequence used in this study, the plant rhizome was first extracted with chloroform, followed by extraction with methanol. Consequently, the terpene content of the methanolic extract should be lower than that of the chloroform extract. This may explain why the spasmolytic potency of the methanolic extract used in this study was lower than that of the chloroform extract. If the methanol extract contains only a minimal amount of terpenes, other compounds distinct from those in the chloroform extract may contribute to its actions. However, to solidify these findings, further analysis to confirm the specific chemical constituents should be performed.

The present study further revealed that the reference antidiarrheal drug, loperamide, also inhibited ACh-induced contraction of the isolated rat ileum. The 3 plant extracts were less potent than loperamide, with the order of potency as follows: Loperamide > chloroform extract > methanol extract > water extract. In summary, these findings reinforce the traditional use of CA as an antidiarrheal and antispasmodic agent as well as a means to aid in the relief of abdominal pain.

The methanol and water extracts of the CA rhizome led to concentration-dependent inhibition of ACh-induced contractions in rat gastric fundus strips. The contraction was entirely abolished by atropine (1 μM). Existing evidence reveals that the muscarinic receptors in the rat gastric fundus are M_1 and M_3, with M_1 receptors being more significant [30]. Conversely, the M_3 receptor appears to be the dominant muscarinic receptor regulating cholinergic contraction in rat stomach smooth muscles [12,31]. Nevertheless, the mechanisms by which M_1 and M_3 receptors induce muscle contractions seem to be similar. Activation of these receptors leads to an increase in intracellular Ca^{2+} through stimulation of the Gq-PLC-IP3 pathway, resulting in the release of intracellular Ca^{2+} and an influx of extracellular Ca^{2+} [30,31]. Similar to the findings in rat ileum, the inhibition of ACh-induced contraction in rat gastric fundus strips by CA's methanol and water extracts might be attributed to compounds such as α-pinene, β-pinene and certain sesquiterpenes. The methanol extract was more potent than the water extract, likely due to its higher content of the aforementioned active components.

The current findings indicate that the water extract at the highest dose exerts a significant effect on KCl-induced contractions of the gastric fundus. The underlying mechanism of inhibition may be consistent with that observed in the ileum, as previously discussed. In the non-stimulated or spontaneous contractions of the gastric fundus, CA methanol and water extracts resulted in a concentration-dependent increase in the force of contraction of the fundus strip. Initiation of spontaneous contraction of the depolarization of the smooth muscle by the propagating impulse generated by the pacemaker, the interstitial cell of Cjal (ICCs). The slow wave is caused by depolarization in smooth muscle cells, and if the cells are further depolarized by the release of acetylcholine from enteric nervous system neurons, a "threshold potential" is created and

causes a rapid influx of calcium into the cells, producing a "spike" potential linked to muscle cell contraction. Thus, the spontaneous contractions consist of a slow wave and spike potential. The contraction force depends on the number of spike potentials and amount of Ca^{2+} entry [32]. This effect of extracts could be attributed to the presence of α- and β-pinene, which have previously been reported to induce contractions in gastric strips [23]. However, the mechanism by which these 2 compounds cause an increase in the spike potential and intracellular Ca^{2+} levels needs to be further explored. Therefore, the influence of the extracts on the gastric fundus strip seems to be dependent on basal tone. Acute toxicity tests of CA rhizome extracts in mice have been reported; the LD50 value for orally administered chloroform and methanol extracts was 3.03 g/kg, whereas that for the water extract exceeded 10 g/kg [33]. Consequently, according to the Globally Harmonized System (GHS) classification, chloroform and methanol extracts may pose a slight hazard, but the water extract is unlikely to present an acute hazard.

Conclusions

In this study, the rhizome extracts of *Curcuma aeruginosa* (CA) displayed anti-motility effects on the small intestine, highlighting their potential use as antispasmodic and antidiarrheal agents and for alleviating the symptoms of irritable bowel syndrome (IBS). As mentioned earlier, the essential oil of this plant has antimicrobial activity against diarrhea. Thus, the extracts can be used to treat both infectious and noninfectious diarrhea, similar to FGIDs. In the ileum, the order of potency of CA rhizome, based on ACh-induced contraction, was chloroform > methanol > water extracts, with respective IC_{50} values of 0.025 ± 0.001, 0.46 ± 0.02 and 2.57 ± 0.47 mg/mL. Similar potency order against KCl-induced contraction was observed, with IC_{50} values of 0.011 ± 0.002, 0.47 ± 0.06 and 1.18 ± 0.18 mg/mL, respectively. In the isolated gastric fundus model, when ACh (0.3 μM) was introduced, the methanol and water extracts exhibited a concentration-dependent reduction in contractions, with IC_{50} values of 0.62 ± 0.06 and 2.30 ± 0.32 mg/mL, respectively. As for KCl (40 mM)-induced contraction, only the highest concentration of the water extract (3.75 mg/mL) significantly decreased the contraction by 41.29 ± 2.94 %. The observed relaxing effect of the extracts appears to be related to the inhibition of voltage-dependent calcium channels and the intracellular release of Ca^{2+}, likely due to the presence of α-pinene, β-pinene, 1,8-cineol and sesquiterpenes components. Additionally, the extract increased spontaneous contractions in rat gastric fundus strips. This suggests their potential as gastroprokinetic agents, which will be useful in the treatment of gastroesophageal reflux disease and gastroparesis. However, before its clinical implications, the extract should be subjected to an *in vivo* study to determine its effects on the gastric emptying rate. Further clinical research and investigations are warranted to establish their overall therapeutic efficacy.

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